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Assessing the effects of Nano Alumina on general body condition and behaviour of white Leghorn chickens: An experimental study

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Abstract
Present study was conducted to understand the affects of nano alumina on different clinical signs and growth parameters in White leghorn chickens. The randomly divided control group was fed with normal standard recommended feed while treated group exposed to nano alumina through oral route in feed for three months. The birds of control group (group I) showed signs of some increased activeness or aggressiveness. At the time of feeding birds from treated group (group II) showed increased response for feeding as compare to group I. There was increased fighting tendency noticed in birds of group II. There was no significant difference in body weight observed between group I and group II throughout the experiment at different time intervals. There was a slight 0.33 %, non significant decrease in the body weight of treated group as compared to controls at 90 days post treatment (DPT).

Keywords: White leghorn chickens, Nano Alumina, clinical signs, growth parameters, organ body weight ratio

Introduction
India has placed at 3rd position after China and America with a production of 66.5 billion eggs and 5th after America, China, Brazil and Mexico with 2.5 million metric tonnes production of chicken meat during 2011-12 (CARI VISION 2050, 2015) [3]. Indian poultry sector with growth of 7.3% in poultry population. Fastest annual growth of about 6% in eggs and 10% in meat production over the last decade amongst all animal based sectors.

Nanotechnology was used by ancient Indians is well discussed by Sir Walter Scott in his book "Talisman". In 'Charak Samhita' the concept of reducing size of particles is well explained. Utmost reduction in particle size of metals and non metals is termed as nanotechnology. Ayurvedic system of medicine used 'bhasmas' of metals encapsulated with herbal molecules to cure various human diseases (Chauhan *et al*., 2010) [4]. In ancient time the goldsmiths were well known for preparing of ornament and also to provide the bhasmas (powder of coal and minerals) for treating different ailments in human beings (Baboo, 2015) [2].

Nanotechnology is the convergence of engineering and molecular biology, leading to the development of structures and systems that have novel functional properties (Seetharam and Sridhar, 2007) [25]. The special physico-chemical properties of engineered nanoparticles are exploited in a broad range of applications as diverse as cosmetics, pharmaceuticals, textiles, electronics, biosensors and catalysts (Handy *et al.*, 2008) [8]. Nanomaterials can be composed of many different base materials (carbon, silicon, and metals such as gold, cadmium, aluminium and selenium). According to Oberdorster *et al.*, 2005 [21], the size of the particles is not only the factor that causes changes in the biological activities of materials at the nanoscale but also the characteristics like; size distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge and also porosity are linked to nanotoxicity. Warheit *et al*., 2007 [29] reported that the toxicity for cytotoxic crystalline quartz did not relate to particle size, but did relate to surface reactivity as measured by hemoglobin release from cells. Particle size and surface area are important material characteristics from a toxicological perspective.

Nanomaterials enter the cells of the organ and reside in the cells for an unknown amount of time before moving to other organs or before getting excreted (Fischer and Chan, 2007) **[6]**. Interaction with biological systems can give rise to toxic effects like allergy

(Maynard *et al.*, 2006) [15], fibrosis, deposition in different organs that can lead to organ failure, inflammation, cytotoxicity (Nel *et al.*, 2006) [20], ROS generation (Meng *et al.*, 2007) [16], tissue damage and DNA damage (Singh *et al.*, 2009) [27]. Interaction of nanoparticles with lymphocytes and other cell types can grant to a varied spectrum of possible impacts, including inflammation, hypersensitivity and immunomodulation (Ambwani *et al*., 2015) [1].

Nanomaterial toxicity can occur through several different mechanisms in the body as summarized by Lanone and Boczkowski, 2006 [10]. The main molecular mechanism of nanotoxicity is the induction of oxidative stress by free radical formation. Studies reported that aluminium oxide nanoparticles could induce oxidative stress via increased generation of reactive oxygen species (ROS) (Li *et al.*, 2012; Prabhakar *et al.*, 2012; Morsy *et al.*, 2016) [12, 24, 17]. Aluminum oxide nano particles have wide-range of application in industrial as well as personal care products in cosmetics. Because of these reasons, their impact on the environment requires a detailed investigation.

Occupational health studies shown that finest aluminum powder can cause pulmonary fibrosis under unfavorable industrial-hygiene conditions. In Germany, the disease aluminosis has been approved as such and workers have been recompensed for the health related problems since 1943 (Kraus *et al.*, 2000) [9]. While concerns about potential risks have been raised with the increasing use of aluminium oxide nanoparticles, their toxicological profile is still unclear (Oesterling *et al.*, 2008; Park *et al.*, 2015) [22, 23]. Keeping in view the above facts, the present study has been planned to know the affects of nano alumina on different clinical signs and growth parameters in White leghorn chickens of two week age

Material and Methods

The research work was conducted at Department of Veterinary Pathology, C.V.A.Sc. and Instructional poultry farm unit (IPF), Nagla, GBPUAT, Pantnagar. The materials used, experimental procedures and techniques that were followed during the course of experimentation are given below.

Procurement and maintenance of experimental chickens

One week old layer chicken were procured from Instructional Poultry Farm, Govind Ballabh Pant University of Agriculture & Technology, Nagla, Pantnagar, Udham Singh Nagar, Uttarakhand, India. The chickens were kept under observation in separate pens at Instructional Poultry Farm, Nagla, and fed standard recommended feed and RO water *ad-libitum* from one day of age until the start of experiment i.e. 2 weeks of age. The chickens were maintained on deep litter system under proper light maintaining good hygienic conditions. The birds were vaccinated with Ranikhet disease at 5th day using F1 strain vaccine (Indovax Pvt. Ltd, Hisar) through oral route. The birds were vaccinated orally for Infectious Bursal Disease at 11th day using live intermediate strain, Bursa B2k (Indovax Pvt. Ltd, Hisar). At 37th day of age, birds were be revaccinated for Ranikhet disease using Lasota strain vaccine (Indovax Pvt. Ltd, Hisar) through oral route. Birds were vaccinated through wing web method for Fowl Pox at 47th day using Fowl Pox vaccine (Indovax Pvt. Ltd, Hisar). Birds were administered a booster dose against Ranikhet disease at 58th day of age using Lasota strain vaccine (Indovax Pvt. Ltd, Hisar) by wing web prick method.

Test Compound

The test compound, Nano alumina, of commercial grade (Sisco Research Laboratories Pvt. Ltd) used in the study was procured from local market. The Nano alumina used as aluminium oxide (Boehmite) with characterization; nano dispersion (50nm), APS (Aerodynamic particle size): 50nm, pH: 4, specific gravity: 1.19, viscosity: 10cps (centipoise), Molecular weight: 59.99.

Dose selection for test compound

The dose for the study was selected based on the maximum tolerable level of alumina in the feed of chickens was reported to be 200 ppm (National Research Council, 1980) [18].

Experimental design

The experiment was conducted on 40 white leghorn chickens. The birds were randomly divided into two groups of 20 birds each to study the pathological effects of nano alumina. First group (G_1) was kept as control and given with normal standard recommended feed. Second group (G_2) was fed with maximum tolerable limit that is 200 ppm (National Research Council, 1980) [18] of nanoalumina in feed from 2 weeks of age of birds till 90 days post treatment (DPT); both treated and control group were supplied with RO water *ad-libitum.*

Clinical signs, Behaviour and Body weight

Chickens were observed daily for manifestation of any clinical signs and behavior throughout the period of experiment i.e. upto 90th DPT.

Body and organ weight

Body weight of each bird was recorded in every two weeks for three months. In addition, at the end of experiment relative and absolute organ weight of vital organs like liver, thymus, spleen and bursa were calculated. Relative organ weight were calculated by given formula.

$$\text{Relative organ weight (\%)} = \frac{\text{Absolute organ weight}}{\text{Total body weight}} \times 100$$

Statistical Analysis

The data generated during the experiment was statistically analysed by using standard statistical procedures (Snedecor and Cochran, 1994) [28] with the help of SPSS software. The collected data was analyzed by one way ANOVA.

Results

The present study was undertaken to study the general effects of nanoalumina in white leghorn chickens at maximum permissible dose after an exposer for three months.

Clinical signs

Chickens of the both experimental groups were monitored daily in the morning for any kind of the clinical manifestation, if any. In chickens of both the groups toxicity signs like abnormal posture paralysis, bleeding, diarrhoea, convulsion, breathing difficulties, restlessness and irritation were not observed. The birds of group I showed signs of some increased activeness or aggressiveness. At the time of feeding birds from group II showed increased response for feeding as compare to group I.

There was increased fighting tendency noticed in birds of group II. There was no mortality throughout the course of the study in either group.

Body weight

Body weight of the chickens from both the groups was taken at different time intervals and is expressed in grams and presented in Table 1 and Fig.1.

There was no significant difference in weight observed between group I and group II throughout the experiment at different time intervals. There was a slight 0.33 %, non significant decrease in the body weight of treated group as compared to controls at 90 DPT.

Table 1: Mean body weight (grams) in different groups of experimental chickens at different time intervals (Mean ± SE)

DPT \ Group	Control	Treated
0 DPT	83.65±3.03	87.30±3.08 (4.36%)
15 DPT	205.70±8.59	208.15±7.12 (1.19%)
30 DPT	313.80±9.29	319.80±9.95 (1.91%)
45 DPT	457.35±19.79	489.90±19.59 (3.18%)
60 DPT	640.50±26.53	657.20±25.97 (2.61%)
75 DPT	851.40±36.42	852.90±38.53 (0.18%)
90 DPT	1036.80±44.58	1033.40±49.85 (-0.33%)

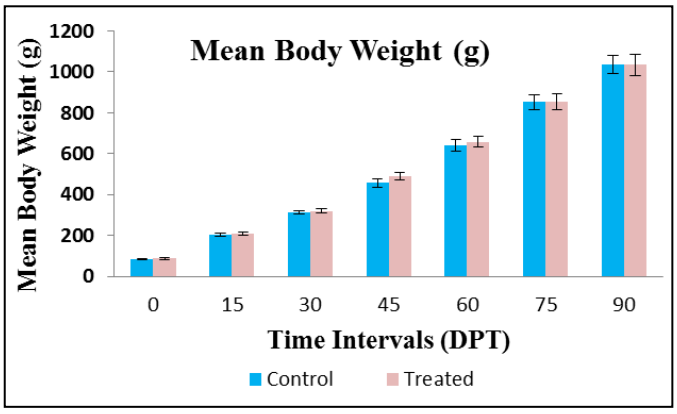

Fig 1: Mean body weight (g) in different groups of experimental chickens at different time intervals

Absolute organ weight

Absolute organ weight of the chickens from both the groups ware expressed in grams and is presented in Table 2 and Fig. 2. There was reduction in weight of the organs viz. liver, spleen, thymus and bursa.

However, weight of liver, spleen and bursa of treated group were not significantly different when compared with control group. The weight of thymus showed significant difference and a decrease of 14.58 % weight as compared to control group.

Treated group also showed a decrease of 9.06 %, 10.72 % and 6.00 % in liver, spleen, and bursa weight respectively as compared to control group at 90 DPT.

Table 2: Mean absolute organ weight (grams) in different groups of experimental chickens at the end of experiment (90 DPT) (Mean ± SE)

Organ \ Group	Control	Treated
Liver	27.60±0.89	25.10±1.82 (-9.06%)
Spleen	3.45±0.18	3.08±0.26 (-10.72%)
Thymus*	3.43^{a}±0.13	2.93^{b}±0.21 (-14.58%)
Bursa	3.33±0.17	3.13±0.27 (-6.00%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

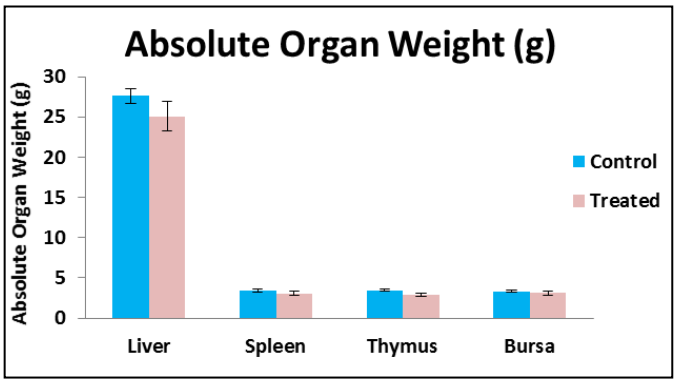

Fig 2: Mean absolute organ weight (g) in different groups of experimental chickens at the end of experiment (90 DPT)

Relative organ weight

Relative organ weight of the chickens calculated at different time intervals from both the groups were is expressed in percentage and presented in Table 3 and Fig. 3. Organ body weight ratio of different organs like liver, spleen, thymus and bursa were reduced by 9.20 %, 9.68 %, 12.90 % and 3.34 % in liver, spleen, thymus and bursa, respectively. However, it did not show significant difference as compared to control groups at 90 DPT.

Table 3: Mean relative organ weight (%) in different groups of experimental chickens at the end of experiment (90 DPT) (Mean ± SE)

Organ \ Group	Control	Treated
Liver	2.5±0.07	2.27±0.12 (-9.20%)
Spleen	0.31±0.01	0.28±0.02 (-9.68%)
Thymus	0.31±0.01	0.27±0.02 (-12.90%)
Bursa	0.30±0.01	0.29±0.03 (-3.34%)

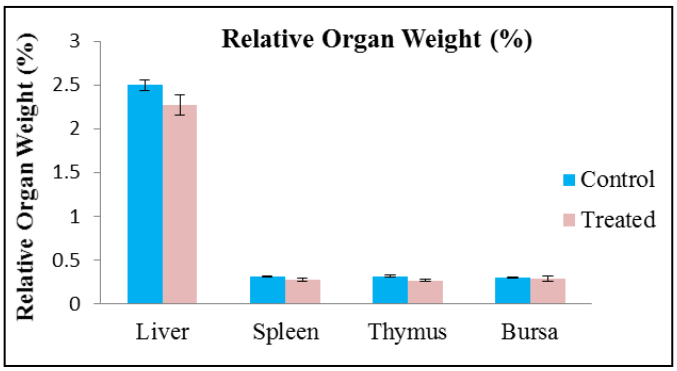

Fig 3: Mean relative organ weight (%) in different groups of experimental chickens at the end of experiment (90 DPT)

Discussion

In this investigation the two week old chickens were given 200 ppm dose of nanoalumina for a period of 90 days and various hematolgical, immunopathological and pathological parameters were studied. The birds of treated group showed clinical signs as increased activeness, quick response to feeding and aggressiveness as compared to birds of control group. It may be due to interruption of neurobehavioral functions. Zhang *et al.*, 2011 [31] and Li *et al.*, 2013 [13] reported that nanoalumina interrupted neurobehavioral functions. Mitochondrial impairment, sequent oxidative damage, neural cell loss and necrosis, may be possible reason for the neurobehavioral alterations.

Results of body weight showed insignificant change when

compared among the treated and control groups. Similar results have been reported by Yang *et al.*, 2012 [30], Li *et al.* 2009 [11] reported that the mean body weight of nanoscale aluminium oxide treated rats were similar to rats of non-nanoscale aluminium oxide and control groups. Nehru and Anand, 2005 [19] reported there was no significant decrease in the body weight of adult animals as compared to their control animals, but a significant decrease in body weight was found in the aluminium treated pup group as compared to their control group. However, Shakoor *et al.*, 2000, Druga *et al.*, 2010, Zhu *et al.*, 2012, Hu *et al.*, 2013 and Luo *et al.*, 2014 [26, 5, 32, 8, 14] recorded significant decrease in body weight of treated group as compared to the control group.

Present study revealed that there was no significant difference observed in the relative organ weight of liver, spleen, thymus and bursa when compared between treated and control groups. Absolute organ weight of thymus showed significant difference while liver, spleen and bursa were not showed significant difference when compared among treated and control groups. Prabhakar *et al.*, 2012 [24] also reported that Al_2O_3 nanomaterials and bulk material caused no significant change in organ weight of treated rats when compared with controls. Luo *et al.*, 2014 [14] reported results that spleen weight were significantly lower (p<0.05) in animals exposed to $AlCl_3$, compared with the control group.

Most changes may be not significant statistically but certainly indicates the pathological effects of nanoalumina. Actually at such a lower dose (maximum permissible dose) it takes time to develop pathological effects. It is therefore, proposed that further studies should be carried out in different animal models using varied doses and increased duration to exactly find out the pathological alterations.

Conclusion

Based on the above findings, it was concluded that nanoalumina causes ill effects on the health status of chickens even at maximum tolerable level. It induces structural and functional alteration in various organs of the body and thus causes health hazards leading losses in terms of production. It is suggested that further studies should be carried out in different animal models using varied doses and increased duration to exactly find out the pathological alterations.

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